

## Antibodies against T cells as therapeutics

The invention relates to antibodies against T cells as therapeutics to prolong immunosuppression and tumor cell elimination.

A rejection of a transplant is treated with immunosuppressants, e.g. monoclonal, immunosuppressive antibodies against human T lymphocytes generated from mice, rats or golden hamsters. However, their effect is limited by an immunoreaction the patient develops to the antibody proteins derived from another animal species. This results in what are called anti-antibodies, which inhibit the immunosuppressive effect of the injected antibodies. Thus, at present e.g. rejection crises of patients with kidney transplants are usually treated with only one single antibody therapy. If another rejection crisis occurs, this treatment is usually not repeated because of a possible formation of anti-antibodies.

So far, there is no clinical therapy of choice for prolonging the immunosuppressive effect of antibodies while avoiding the formation of anti-antibodies. A repeated treatment with another monoclonal antibody also leads to a - possibly even accelerated - formation of anti-antibodies [1]. Patients also developed anti-antibodies against immunosuppressive antibodies that had been humanized applying genetic engineering methods, i.e. they had been substantially adapted to the patients' "primate species" or "species-adapted" [2].

Experimentally, a clear prolongation of the survival time of skin transplants was found in the mouse model, which was even considered a tolerance induction. It was observed after the injection of high doses of a rat antibody pair directed to mouse T(L3T4+Lyt-2) cells followed by a second antibody pair of the same species and the same cell binding specificity, which, however, differed from the first by its low elimination of T cells from the blood circulation of the mouse (= "non-depleting" i.e. eliminating antibody pair). Unlike the present invention, the described principle of action is not based on a combined therapy of at least 2 antibodies with species-different Fc regions [3].

Prolonged survival time of skin transplants and lack of formation of anti-antibodies were furthermore found after the injection of a rat anti-mouse T(L3T4 = CD4+ lymphocyte subpopulation)-cell antibody followed by injection of (Fab')<sub>2</sub> fragments and unfragmented monoclonal hamster anti-mouse T(CD3) antibodies [4]. Here, too, the described principle of action is not based on a combined therapy of two antibodies having species-different Fc regions that are directed to all T cells as in the invention but rather on the suppression of the CD4+T lymphocyte subpopulation [5] achieved by means of the first antibody, which is, however, not sufficient.

By means of a different method, which, however, involves risks, permanent tolerance of skin transplants can be achieved in irradiated mice after transplantation of bone marrow of the donor of the skin transplant and while protecting anti-T cell antibodies [6].

So far, there is no therapy of choice for definitely preventing the formation of anti-antibodies in the patient in case of conventional poly- or monoclonal immunosuppressive antibodies. The first clinical experiences with antibodies that have recently been humanized by means of genetic engineering show that anti-antibodies may be formed [2] similarly to what was shown with murine immunosuppressive anti-mouse T cell antibodies [7].

A combination of immunosuppressive antibody treatment with chemotherapy, e.g. cyclophosphamide or busulfan, involves the risk of side effects particularly on hematopoiesis but also on the transplanted tissue due to lack of cell specificity of chemotherapeutic agents [3, 8].

The object underlying the invention is to provide antibodies for a clinical therapy for prolonging the immunosuppressive antibody effect while avoiding the formation of anti-antibodies.

This object is achieved by the features of claim 1.

The subclaims describe advantageous embodiments of the invention.

Each of the two groups A, B of antibodies may also consist of only one antibody type or several kinds of antibodies. Groups A, B may also be monoclonal or polyclonal. The sequential treatment with anti-T cell antibodies that were partially or fully humanized applying molecular biological means and non-humanized - or with at least two anti-T cell antibodies generated from different species - depicted here leads to a prolonged immunosuppression and tumor cell suppression. It is a treatment principle that was experimentally tested on animals and has not been described yet.

The novel therapy principle of the invention, which has not been found and/or published by other authors yet, is not obvious at all in terms of immunology. Commonly, immunobiologists and experts in the field of medicine search for a reduction of the immunogenicity of anti-T cell antibodies (which causes the formation of anti-antibodies) in as much an adaptation thereof as possible to the patient's antibody immunoglobulin structures so that s/he is more likely to tolerate them, e.g. by humanization by means of genetic engineering of the monoclonal immunosuppressive antibodies derived from mice. However, principally this adaptation cannot possibly be complete and is the cause for the formation of anti-antibodies because the T cell binding (V) region of the immunosuppressive antibody is so variable that the patient's immunoapparatus can still form anti-antibodies thereto.

The invention is rather based on a contrasting experience, namely on the suppression of anti-antibodies by creating a high species difference of anti-T cell antibodies, of which one or both, applied alone, may be potentially immunogenic in the recipient of the antibodies.

It was found that

- a) the survival time of skin transplants was basically prolonged not by applying two different mouse anti-mouse T cell antibodies (or two rat anti-mouse T

cell antibodies) after one another, but by the fact that two antibodies which are species-different to one another but not necessarily to the recipient of the antibodies lead to a clear prolonged immunosuppression

- b) two antibodies are effective even when they are as different from one another as human and mouse.

These thoughts, results and antibody combinations define a therapy model offering *inter alia* the advantage that it can immediately be tested clinically and does not expose the patients to any additional treatment risks. A prolongation of immunosuppression should not only be a more successful therapy for rejection crises of organ transplants and immune complications with bone marrow transplantations but should help prevent them altogether by prophylactic treatment. In addition, autoimmune diseases, chronic diseases of all kinds of rheumatism but also individual tumor conditions might face new therapeutic perspectives. For instance, in the mouse model studies carried out by the inventors on the suppression of murine or human T cell leukemias transplanted onto mice show a prolonged survival time due to antibody injection. Upon T cell depletion, foreign immunocompetent cells can be introduced in chimerical mice, i.e. mice transplanted with bone marrow and suffering from leukemia, which foreign immunocompetent cells attack the neoplastic cells in the recipient. Furthermore, the tolerance induction vis-à-vis heterologous serum protein described under b) makes a passive vaccination with antibodies of a different species possible that is free of hypersensitive reactions, e.g. for tetanus.

In the murine skin transplant model it could be shown that a monoclonal, immunosuppressive antibody that was humanized by genetic engineering methods achieves a survival time of transplants to the murine T lymphocytes that is prolonged by a multitude when its application was preceded by one or more injections of a monoclonal immunosuppressive mouse antibody. The preceding antibody injections as such did not have to be immunosuppressive in the sense of a transplant prolongation. It turned out that this antibody therapy induced a complete tolerance towards heterologous, human serum protein, which still

remained five months after the end of the immunosuppressive therapy. The unexpected prolongation of the immunosuppressive effect was thus accompanied by a lack of a formation of anti-antibodies in the treated mice due to their tolerance of the heterologous antibody immunoglobulin. The principle of action underlying this phenomenon is analyzed particularly with regard to species-related differences in the Fc region of the combined antibodies. It also proved effective when anti-T cell antibodies that had not been molecularbiologically modified if they were species-different to one another.

Antibodies have what is called a variable region that includes the antibody binding site and what is called a constant Fc region that mediates antibody effector functions (e.g. elimination of body cells occupied by antibodies from the system), which is located on what are called the constant regions of the heavy chains of the antibody. This way two antibodies can be similar with regard to their specificity to bind e.g. human T lymphocytes. Such antibodies with the same cell binding specificity, however, may differ in their Fc region due to the fact that they stem from different normal or molecularbiologically manipulated animal species. They can also be modified in vitro in the Fc region using methods of molecular biology upon generating antibody-secreting cells (e.g. hybridomas or hybrid hybridomas) so that a degree of difference is obtained as there is between human and rodent and is described in the invention.

In the following, the invention is described in more detail based on three examples and using the Figures.

Figs. 1, 2 and 3 each show a test report in the form of a diagram of Examples A, B and C.

Fig. 1 (Example A) shows an immunosuppression that was prolonged approximately ten-fold measured in a rodent skin transplantation model of maximum histoincompatibility. It was achieved by combined Fc-region incompatible antibody treatment. In the MmT1/T23 antibody treatment, an injection of a mouse anti-mouse T cell antibody (MmT1[7]) was at first applied,

followed by the T23 antibody twice a week, which differs from MmT1 by an exchange of the murine for a human IgG1 Fc region achieved by means of genetic engineering. A single dose of MmT1 did not prolong the (average) skin survival time. T23 alone, applied twice a week did prolong it by nine days from 16 to 24, MmT1 (first dose) followed by T23 (applied twice a week) prolonged it to more than 90 days. A similarly increased immunosuppression was also achieved after replacement of MmT1 by MmT5 [7], which does not differ from MmT1 in its T-cell specificity but rather in the microstructure of the antibody binding site (idiotype). This results in the conclusion that in the combined Fc-region incompatible antibody therapy, the likeness or difference of the antibody binding site is not a prerequisite for the principle of action but rather the species-dependent difference of its heavy chains incorporating the Fc regions.

Fig. 2 (Example B) shows that rat anti-mouse T cell antibodies having Thy-1 specificity [9] and also particularly clinic-relevant antibody specificities such as anti-CD4 and anti-CD8 (2 T cell subpopulations, which together bind all T cells) also prolong the average survival time of skin transplants by preinjection of one dose MmT1 in the MmT1/RmCD4+CD8 combination. Furthermore, it is shown that the reversal of the combined antibody treatment in the RmCD4+CD8/MmT1 combination also leads to a prolonged immunosuppression of MmT1 since here, too, the prerequisite of the species-dependent difference of the Fc region is fulfilled. Since anti-CD4+CD8 are active as first antibodies, this excludes an effect of the preinjected first antibody restricted to MmT1. On the contrary, the prerequisite for the synergistic antibody action of their species-dependent Fc region differences (apart from the application of at least two antibodies at different times) applies again and again: A survival of skin transplants together with a continued antibody therapy was finally permanent if further T cell depleting and/or T cell receptor modulating (anti-CD3) antibodies were added to the second antibody.

Fig. 3 (Example C) uses Example B to show that the combined rat/mouse or mouse/rat Fc-region incompatible antibody treatment leads to a high suppression

or complete lack of the formation of antiantibodies. The same applies to Example A.

Antiantibodies also occur in the treatment with polyclonal antibodies that occur after immunization of e.g. rabbit, rat or horse lymphocytes; here, too, it can be seen that a species difference (Example rabbit/rat) of polyclonal antibodies leads to a prolonged immunosuppression in mice, as well as with what are called bispecific antibodies, i.e. antibodies having two different binding sites or with anti-T cell antibodies that were chemically modified by introduction of a low-molecular compound (e.g. DNP, TNP haptens) or by genetic engineering, e.g. antibodies and antibody fragments prepared in bacteria. Here, too, sequentially injected anti-T cell antibodies may neutralize in the formation of antiantibodies to form species-different polyclonal or bispecific or chemically or molecularbiologically modified antibodies. A prerequisite is always a strong difference of the sequentially applied antibodies or antibody groups as it either results from species difference or from the introduction (conjugation) of chemical compounds.

Finally, undesired immunoreactions may also occur in case of passive immunization with antibodies in protein-oversensitive or presensitized patients, where a preventive treatment prevents the formation of antiantibodies by combined Fc-region incompatible antibody therapy.

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